THE IMMUNOGLOBULINS OF MICE

V. The Metabolic (Catabolic) Properties of Five Immunoglobulin Classes

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Antibodies of all species are present in several classes of immunoglobulin. The serum level of each immunoglobulin class is determined by its rate of synthesis and catabolism. Thus catabolic properties are important biologic features of antibody because of the importance of catabolism in regulating serum level and in determining the survival of an antibody molecule.

Studies in man (1, 2), rabbit (3), and guinea pig (4) have shown that the 18S γ_1-macroglobulins (IgM) are catabolized much more rapidly than 7S γ_2-globulins (IgG). Thus, immunoglobulin classes may differ in their rates of turnover. Differences in the catabolic control of immunoglobulins are also discernable. The serum level of 7S γ-globulin (IgG) helps to determine the catabolic rate; the higher the serum level, the greater the catabolic rate (5, 6). 18S γ_1-macroglobulin (IgM) catabolism, however, appears to be unrelated to the serum γ_1-macroglobulin level (2). Thus the catabolic properties of all classes of immunoglobulins need to be characterized and the catabolic interrelationships between immunoglobulin classes further defined.

Four major classes of immunoglobulins, the 7S γ_2-globulins, the 7S γ_1-globulins, the γ_1A (β_2A, IgA)-globulins and γ_1M (IgM)-macroglobulins have been identified in recent studies in mice (7). These components are seen on immunoelectrophoresis of hyperimmune serum in Fig. 1. In addition, two subclasses of 7S γ_2-globulin have been identified and tentatively designated as γ_{2a}- and γ_{2b}-globulins (8). These findings provided an opportunity to investigate further the catabolic relationship between different classes of immunoglobulin. In no species has the metabolism of five classes of immunoglobulin been compared. The present investigation was undertaken to compare the rate of catabolism of γ_{2a}-, γ_{2b}-, and 7S γ_1-globulins, γ_1A (β_2A)-globulins, and γ_1-macroglobulins in mice. Investigations were carried out in mice with low, normal, or high levels of all immunoglobulins, as well as in mice with selective immunoglobulin increases produced by plasma cell tumors.

Materials and Methods

Mice.—White Swiss-Webster (NIH-WS) mice were obtained from the general purpose supply colony of the National Institutes of Health, Bethesda. Germfree and conventional low pathogen white Swiss-Webster mice were classified on the basis of potential environmental exposure to bacteria (9), and have subnormal serum immunoglobulin levels (Fig. 1). A group of

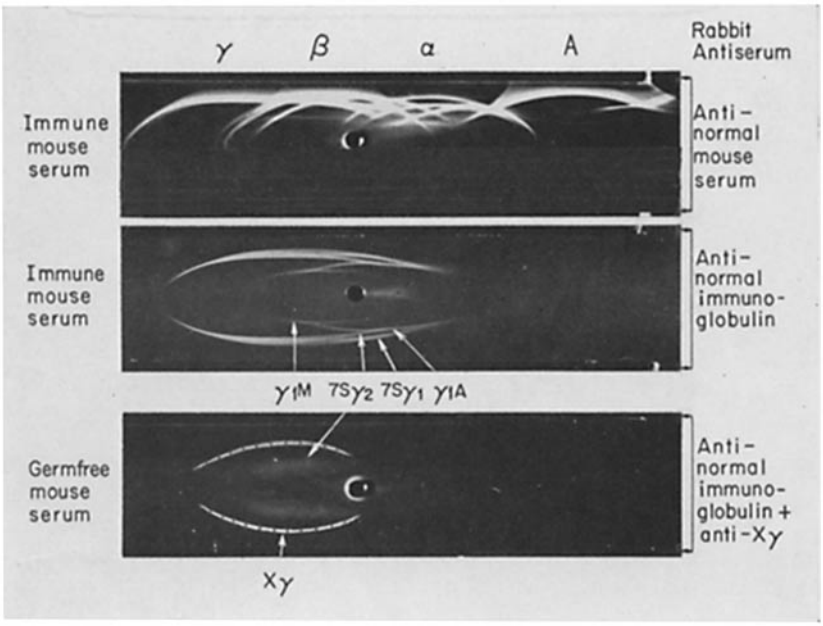

FIG. 1. Immunoelectrophoresis of hyperimmune and germfree mouse serums. Multiple serum proteins are seen when polyvalent rabbit antiserum prepared against whole mouse serum is used to test hyperimmune mouse serum (top figure).

The 7Sγ_2-globulins, 7Sγ_1-globulins, $\gamma_{1A}(\beta_{2A})$-globulins and 18S γ_{1M}-globulins are clearly evident in hyperimmune mouse serum when antiserum against immunoglobulin components is used (middle figure). All immunoglobulins are markedly reduced in germfree mouse serum (bottom figure).

In addition to the immunoglobulins, a component of gamma globulin electrophoretic mobility was detected (Xγ) which is believed to be unrelated to the immunoglobulins (7). The Xγ is also present in normal and hyperimmune serums but is largely concealed within the precipitin arc of the 7S γ-globulins.

six NIH-WS mice were immunized with a total of 0.6 mg of alum-precipitated hemocyanin given in six doses.

C3H/HeN or BALB/c mice harboring nine individual plasma cell tumors were used in these studies. The tumors produced γ_{2a}-myeloma proteins (5563, Adj.PC-5), γ_{2b}-myeloma proteins (MPC-11, MPC-31, MPC-37), or γ_1A-myeloma proteins (SPC-1, MPC-1, MPC-36, MPC-40). Tumor weights were 3 to 7 gm at the time turnover studies were performed.

I^{131}-Labeled Proteins.—Nine individual myeloma proteins were isolated from the sera of tumor-bearing mice by block electrophoresis and column chromatography. Each preparation

was tested by double diffusion in agar against potent rabbit antisera, both specific and polyvalent, to determine the type of protein present and its purity. The nine myeloma proteins included the following: γ_{2a}- (5563 and Adj. PC-5); γ_{2b}-(MPC-11, MPC-31, and MPC-37); 7S γ_1-(MPC-25); and γ_1A-(MPC-1, MPC-40, and SPC-1). The preparations were separately trace labeled (< 1 mole I per mole of protein) with I^{131} by the iodine monochloride method of McFarlane (10). In addition a preparation of human γ_1-macroglobulin from a patient with Waldenström's macroglobulinemia was also trace labeled with I^{131}.

Experimental Protocols.—All animals were housed in plastic cages containing wood shavings that were frequently changed or in cages with wire floors which allowed urine and fecal droppings to pass through to a tray below. All animals were given drinking water containing 0.45 per cent NaCl and 0.01 per cent KI. Mice were injected intraperitoneally with from 0.2 to 1.0 μc of I^{131}-labeled protein in 0.1 ml 0.85 per cent NaCl. The whole body radioactivity of the injected mice was measured in a gamma ray bulk spectrometer (Sharpe Laboratories, La Jolla, California) for 1 minute. The radioactivity in each mouse was determined within 30 minutes of injection, then once every 1 to 2 days. A radioactive standard for each labeled protein was prepared by injecting 1 dose of the given protein into 20 ml of 0.85 per cent NaCl in a 30 ml plastic bottle. This standard was counted daily with the experimental animals and the results used for correction of physical decay. The fractional rate of catabolism (T ½) of each of the I^{131} proteins in each animal was determined from the graphic plots of the decay curves corrected for physical decay of the isotope. The percent I^{131} protein in the body degraded per day was obtained by dividing the T ½ into 0.693.

γ_1-Macroglobulin Catabolism.—γ_1-Macroglobulin catabolism was followed by determining the serum hemolysin titers of mice that were injected with serum or a serum macroglobulin fraction containing sheep cell hemolysin activity. γ_1-Macroglobulin hemolysin was obtained from NIH-WS and C57B1/6JN mice immunized by intraperitoneal injections of 0.2 cc of a 50 per cent suspension of washed sheep cells every 2 to 3 days for 5 weeks. The mice were bled from the retroorbital venous sinus at 3, 4, and 5 weeks after the start of immunization. Each bleeding was done 3 days following the preceding sheep cell injection. The serum obtained from each animal was tested for hemolysin titer by the micromethod described below, before and after incubation with an equal volume of 0.1 M 2-mercaptoethanol. The hemolysin activity of the 3rd week bleedings of the NIH-WS mice and the 3,4,and 5 week bleedings of the C57B1/6 mice was completely destroyed by 2-mercaptoethanol indicating that all of the antibody present was most likely 18S macroglobulin. The hemolysin titers of the sera from the 4th and 5th week bleedings of the NIH-WS mice were only partially reduced following mercaptoethanol treatment, indicating that both 7S and 18S antibodies were present. Serums containing high titers (1:1280 or greater) of mercaptoethanol sensitive antibody were pooled and aliquots injected intravenously into NIH-WS mice. In addition, 2.0 ml of high titered mouse hemolysin serum pooled from the 4th and 5th week bleedings of the NIH-WS mice were passed through a 2.2 × 78 cm sephadex G-200 column. The eluate in the ascending portion of the first protein peak was pooled to obtain the macroglobulin fraction. The pool was concentrated by ultrafiltration until a hemolysin titer of 1:2500 was obtained. The hemolysin activity of this preparation was completely removed by mercaptoethanol treatment. Aliquots (0.2 ml) were injected intravenously into NIH-WS mice and the titers in each mouse followed by the microhemolysin method.

Microhemolysin Technique.—The hemolytic activity of complement (C′) or hemolysin is related to the concentration of sensitized erythrocytes used in the test system (11). In order to follow passively transferred 18S hemolysin activity in mice for more than 1 day, it was necessary to use a system with very small numbers of erythrocytes. The hemolysin method described previously (12) was modified by the use of the microtiter technique (13) for making serial dilutions and the erythrocyte counting method of Sterzl and Kostka (14) for estimation

of the serum dilution giving 50 per cent hemolysis. 0.025 ml of a 1:100 dilution of normal mouse serum in veronal buffered saline (12) was added to each well of a plastic plate. The sera to be tested were then diluted with wire loops, 1 drop of a 0.01 per cent sheep cell suspension added to each well, followed by 1 drop of a 1:30 dilution of normal guinea pig serum (C′). The plates were incubated for 1 hour at 37°C and the reaction then stopped by the addition of 1 drop of isotonic citrate saline solution (1 part 0.075 M aqueous sodium citrate, 4 parts 0.15 M NaCl) (15).

The number of cells remaining in wells spanning 50 per cent lysis were counted microscopically by adding a sample from the appropriate wells to an erythrocyte counting chamber. The dilution of a given serum giving 50 per cent lysis was determined from the intersection of the 50 per cent lysis point by the linear graph of the cell counts of the given wells plotted against the appropriate serum dilutions.

The 50 per cent lysis dilutions from the serial bleedings of a given animal were then plotted against the time following antisera transfer. The rate of catabolism (T ½) of the passively transferred γ_1-macroglobulin antibody was estimated from the graphic plot of the 50 per cent lysis values.

TABLE I

Turnover of Several Classes of Immunoglobulins

Mice	Mean serum immunoglobulin levels			Half-lives of individual I^{131} immunoglobulins*								
				γ_{2a}		γ_{2b}			7S γ_1	γ_{1A} (IgA)		
	γ_2 (a + b)	7S γ_1	γ_{1A}	5563	RPC-5	MPC-11	MPC-31	MPC-37	MPC-25	MPC-1	MPC-40	SPC-1
	(*mg/ml*)	(*mg/ml*)	(*mg/ml*)									
Normal (NIH-WS)	4.0	2.5	0.4	4.8	5.4	2.6	3.0	2.5	4.0	1.3	1.0	1.0
Germfree (GF)	2.0	0.2	Trace	8.2	6.2	2.7	1.8	2.5	14.0	1.2	1.2	1.1
Low-pathogen (CVN-LP)	0.5	0.3	0.25	11.8	10.2	3.3	2.1	5.5	14.0	1.2	1.1	1.1
Hyperimmune (NIH-HI)	15.5	19.5	0.4	2.6	2.2	1.4	2.1	1.1	1.9	—	1.3	1.2

* Mean value of observation in 4 to 6 mice.

RESULTS

Normal Mice.—The catabolism of nine I^{131}-labeled mouse myeloma proteins, representing the 7S γ_{2a}-, 7S γ_{2b}-, 7S γ_1-, and γ_1A (IgA)-globulins, was measured in NIH-WS mice. The mean half-life of each myeloma protein is given in Table I. Representative decay curves for each immunoglobulin subgroup are shown in Fig. 2.

7S γ-globulins: The 7S γ_{2a}-, 7S γ_{2b}-, and 7S γ_1-globulins differed in their catabolic rates. The two γ_{2a}-globulins had the longest mean half-lives (4.8 and

5.4 days) of the 5 classes of mouse immunoglobulins (Table I). The three γ_{2b}-globulins had more rapid rates with half-lives of 2.5, 2.6, and 3.0 days. The half-life for the single 7S γ_1-myeloma protein available for study was 4.0 days.

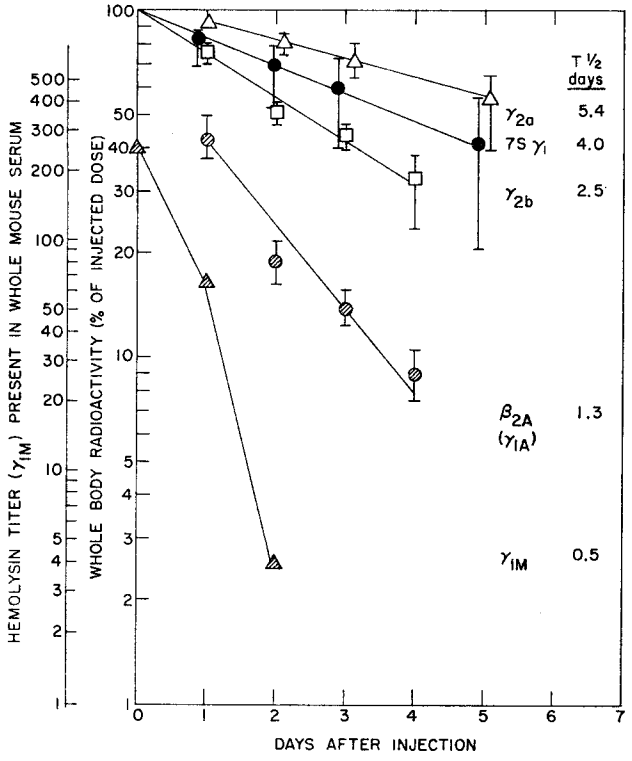

FIG. 2. Catabolism of immunoglobulins (γ_{2a}, γ_{2b}, 7Sγ_1, γ_{1A}(β_{2A}), and γ_{1M}) in normal mice. Median catabolic decay curves of radioiodine-labeled γ_{2a}-, γ_{2b}-, 7Sγ_1-, β_{2A}- and γ_{1M}-globulins in groups of six mice are represented by different symbols. The range of observed data is indicated by brackets.

These observations indicate that 7S γ-globulins are not uniform in their catabolic characteristics. The 7S γ_{2a}-globulins were catabolized at the rate of about 14 per cent per day. The 7S γ_{2b}-globulins are catabolized at rates of 23 to 28 per cent each day; *i.e.*, almost twice as fast as γ_{2a}-globulins.

γ_1A (β_2A, *IgA*)-*globulins:* Three γ_1A-myeloma proteins had half-lives of 1.0, 1.0, and 1.3 days in normal mice. It is possible that the catabolic rate of the γ_1A-globulin is actually more rapid if the observed half-time reflects accumulation in the body of I^{131}, freed from catabolized protein, as well as non-catabolized labeled protein.

γ_1-Macroglobulin (IgM): Metabolism was measured by following the serum hemolysin titer after injection of mouse anti-sheep erythrocyte hemolysin antibodies in whole serum or in macroglobulin fractions prepared by sephadex G-200 filtration of immune serum. Data for a number of individual mice are shown in Fig. 3. Two patterns of decay in serum antibody activity were observed in both normal and low pathogen mice. In some mice a rapid rate of antibody removal was detected with a half-time of 0.2 days. In other mice the initial portion of the decay curve had a half-time of 0.6 days, but on the

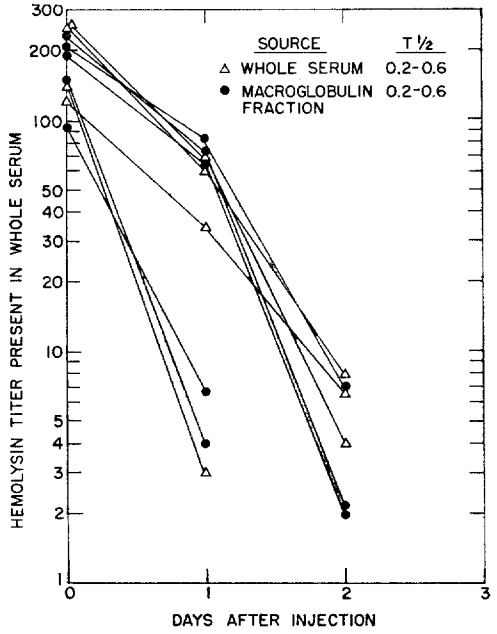

FIG. 3. Catabolism of 18S γ-macroglobulin hemolysin in low pathogen mice.

2nd day the rate of antibody removal was more rapid and half-time of 0.2 days was observed.

Studies with I^{131}-labeled human 18S γ_1-macroglobulin revealed a half-time of 1.3 days for this protein. The half-time is based on whole body radioactivity measurements and calculation of the half-time from daily measurements of the per cent of the injected dose remaining in the whole body. The difference between this half-time (1.3 days) and that observed for the hemolysin antibody (0.2 to 0.6 days) may be due to delayed excretion of I^{131} and retention in the body fluids after catabolism of the protein or may be due to species differences in macroglobulin catabolism.

Germfree and Low Pathogen Mice.—Mice raised in a germfree or low patho-

gen environment have low levels of all mouse immunoglobulin components (Fig. 1, Table I).

The catabolism of each of the 7S globulins was prolonged in the low pathogen and germfree mice as shown in Table I. The comparative data for low pathogen mice are illustrated in Fig. 4 where the half-time of 7S γ_{2a}-globulin was found to be 10 days; 7S γ_{2b}-globulin was 5.5 days; and 7S γ_1-globulin was 14 days. In these mice, as in normal mice, the γ_{2b}-globulins were catabolized more rapidly than γ_{2a}-globulins.

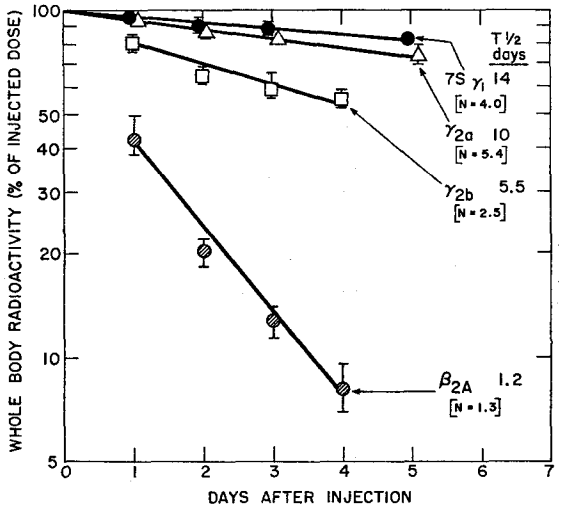

FIG. 4. Catabolism of immunoglobulins, γ_{2a}, γ_{2b}, 7Sγ_1, and $\gamma_{1A}(\beta_{2A})$ in low pathogen (CVN-LP) mice. Catabolic decay curves of the radioiodine-labeled immunoglobulins in mice with subnormal numbers of intestinal microorganisms (CVN-LP) are shown. The T ½ values for the catabolism of γ_{2a}-, γ_{2b}-, 7Sγ_1-, and β_{2A}-globulins in normal mice (N) are given in brackets below the data obtained in this experiment with low pathogen mice.

The rates of catabolism of the γ_1A-myeloma proteins were the same in the mice with low immunoglobulin levels as in normal mice. The half-times for γ_1-macroglobulin antibody (0.2 to 0.6 days) and for I^{131} human macroglobulin (1.3 days) also were the same in low pathogen (CVN) as in normal mice.

Hyperimmune Mice.—In hyperimmunized mice the serum levels of all the immunoglobulin components were increased (Table I). The half-times for the 7S γ_{2a}-globulins (2.2 and 2.6 days), the 7S γ_{2b}-globulins (1.1, 1.4, and 2.1 days), and the 7S γ_1-globulin (1.9 days) were shorter when injected into hyperimmunized mice than in normal mice. The rate of catabolism of γ_1A (IgA)-globulin was not altered in the hyperimmunized mice.

Effect of Specific Immunoglobulin Increases.—Plasma cell tumors synthesize only one class of immunoglobulin, and mice bearing plasma cell tumors have

TABLE II

Immunoglobulin Turnover in Tumor-Bearing Mice

Recipient of I^{131}-labeled proteins	Mean serum immunoglobulin levels			Half-life of individual I^{131} immunoglobulins (days)								
				7S γ_{2a}		7S γ_{2b}			7S γ_1	γ_{1A} (IgA)		
	7S γ_2 a + b	7S γ_1	γ_{1A} (IgA)	5563	Adj. PC-5	MPC-11	MPC-31	MPC-37	MPC-25	MPC-1	MPC-40	SPC-1
	(*mg/ml*)	(*mg/ml*)	(*mg/ml*)									
Normal mice:												
NIH-WS	4.0	2.5	0.4	4.8	5.4	2.6	3.0	2.5	4.0	1.3	1.0	1.0
Tumor bearing mice:												
7Sγ_{2a} 5563	44.0	0.6	0.24	—	—	—	1.6	—	—	—	0.9	0.8
Adj. PC-5	17.7	1.1	0.18	2.2	2.5	—	—	1.0	2.1	1.2	—	—
7S γ_{2b}												
MPC-11	64.7	1.5	0.73	2.2	2.2	1.8	1.0	0.7	1.8	1.2	0.7	0.7
MPC-31	15.3	1.2	0.36	1.8	1.8	1.7	1.1	0.8	1.8	1.2	—	—
MPC-37	23.2	1.4	0.49	1.5	1.8	1.4	1.8	1.0	2.0	1.2	1.0	1.0
γ_1A (IgA)												
MPC-1	1.0	1.3	50.8	3.4	5.0	2.9	2.8	2.0	3.0	1.2	1.1	1.1
MPC-40	1.2	2.3	75.8	4.5	4.3	2.5	—	2.1	3.5	1.2	—	—
MPC-36	0.5	1.1	22.1	—	—	—	3.3	3.1	—	—	1.2	1.3
SPC-1	1.6	1.6	63.5	—	—	—	—	—	—	—	1.0	1.0

a selective increase in serum concentration of the myeloma protein formed by the tumor (Table II). Because of this selective increase, the effects of large amounts of one protein on the catabolism of this protein and other immunoglobulins may be studied (Table II). Mice bearing a γ_{2a}-globulin–producing plasma cell tumor (Adj.PC-5) had a high level of 7S γ_2-globulin (Table II) and showed very rapid catabolism of the γ_{2a}-myeloma protein (T ½ = 2.5 days,

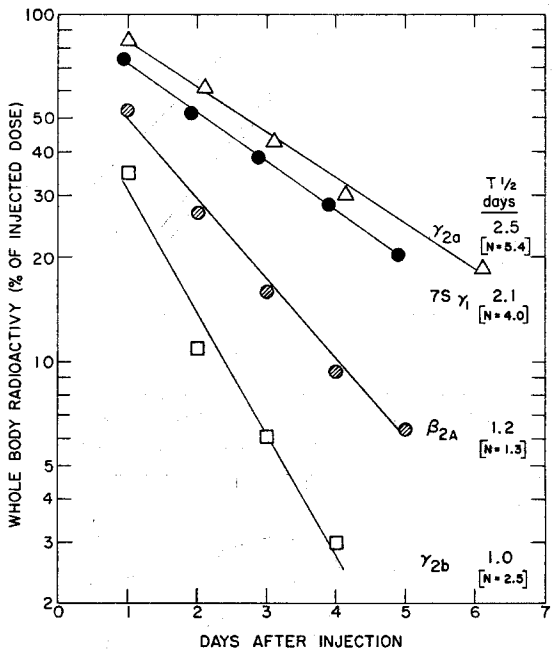

FIG. 5. Catabolism of immunoglobulins, γ_{2a}, γ_{2b}, 7Sγ_1 and $\gamma_{1A}(\beta_{2A})$ in mice bearing γ_{2a} myeloma protein. The catabolic curves of I^{131}-labeled γ_{2a}-, γ_{2b}-, 7S γ_1-, and β_{2A}-globulins in a group of mice bearing plasma cell tumor Adj.PC-5–producing γ_{2a}-myeloma protein are indicated. The T ½ values for the catabolism of each immunoglobulin in normal mice (N) is given in brackets.

in contrast to the normal value of 5.4 days) (Fig. 5). Not only was the catabolism of γ_{2a}-globulin accelerated but also the catabolism of γ_{2b}-globulin and 7S γ_1-globulin was increased by the high serum γ_{2a}-globulin level (Fig. 5). The γ_1A-globulin catabolism, however, was unaltered by large quantities of γ_2-myeloma protein (Fig. 5) (Table II).

Large quantities of γ_{2b}-myeloma protein accelerated the catabolism of all three classes of 7S γ-globulin (Table II). The turnover of γ_{2a}-globulin, γ_{2b}-globulin, and 7S γ_1-globulin in mice bearing γ_{2b}-type plasma cell tumor MPC-37 is shown in Fig. 6, where they are seen to be catabolized at a much greater

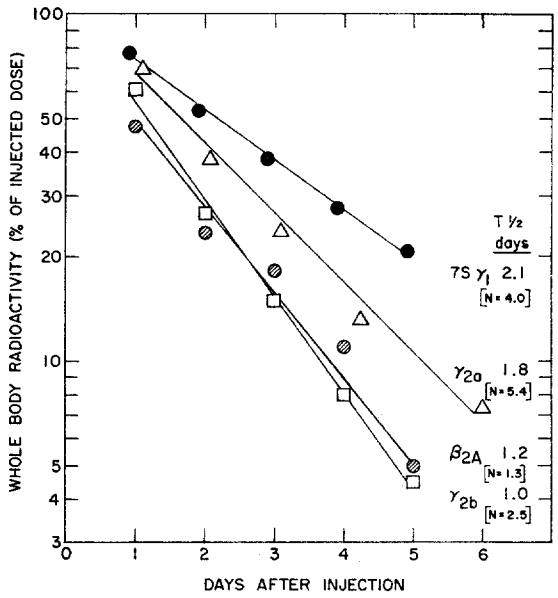

FIG. 6. Catabolism of immunoglobulins, γ_{2a}, γ_{2b}, 7S γ_1, and $\gamma_{1A}(\beta_{2A})$ in mice bearing γ_{2b}-myeloma protein. The catabolic curves for each I^{131}-labeled immunoglobulin in a group of three mice with plasma cell tumor MPC-37–producing γ_{2b}-myeloma protein is shown.

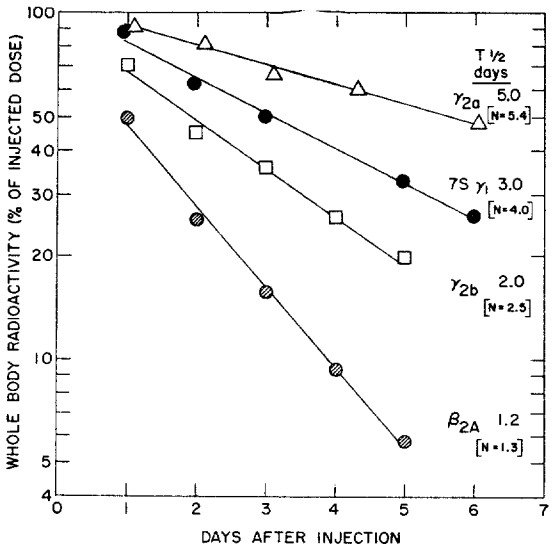

FIG. 7. Catabolism of immunoglobulins, γ_{2a}, γ_{2b}, 7S γ_1, and $\gamma_{1A}(\beta_{2A})$,- in mice bearing β_{2A}-myeloma protein. Catabolic curves for I^{131}-labeled γ_{2a}-, γ_{2b}-, 7S γ_1-, and β_{2A}-globulins in a group of mice bearing plasma cell tumor MPC-1 which produces a $\gamma_{1A}(\beta_{2A})$-myeloma protein are indicated. The catabolic rate for each immunoglobulin in normal mice (N) is given in brackets.

rate than in normal mice. Similar observations were made with γ_{2b}-type tumors MPC-11 and MPC-31 (Table II).

The catabolism of γ_1A (IgA)-globulins on the other hand appeared to be unaffected by a large quantity of γ_{2b}-globulin. Mice bearing 7S γ_1-globulin–producing tumors were not available for study so that a direct comparison of

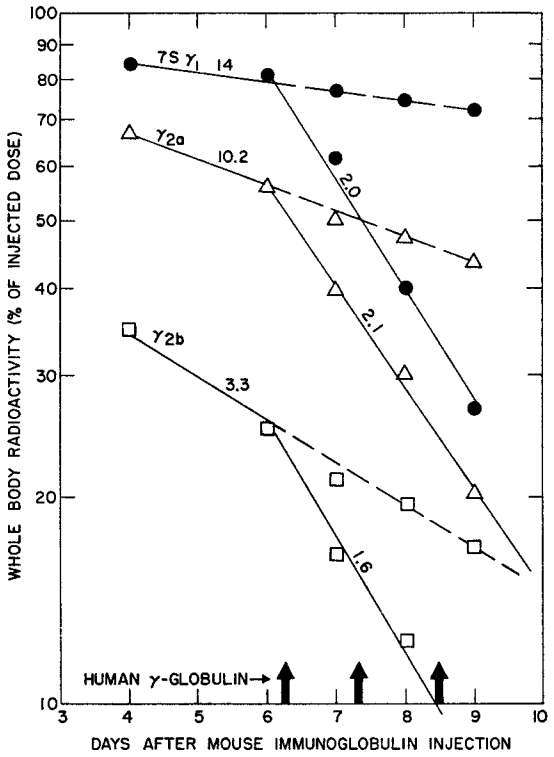

FIG. 8. Effect of exogenous (human) γ-globulin on the catabolism of individual mouse 7S immunoglobulins. Low-pathogen mice were used. Six mice were injected with each protein. Three served as controls and the remaining three in each group were injected intraperitoneally with human γ-globulin (50 mg on day 6, and 10 mg on days 7 and 8).

the effect of these tumors on each of the immunoglobulin components could not be made.

Large amounts of γ_1A (IgA)-myeloma protein did not have any appreciable effect on the catabolism of 7S γ-globulin components (Fig. 7). Each of the 7S γ-globulin components was catabolized at a rate close to normal in mice bearing γ_1A (IgA)-type plasma cell tumors (Table II). γ_1A-globulin catabolism was also unaltered by the presence of large amounts of γ_1A-myeloma protein (Table II).

Effect of Exogenous 7S γ-Globulin.—Prior studies (5, 16, 17) have shown that

the administration of human 7S γ-globulin to mice accelerates the catabolism of normal 7S γ-globulin of mouse origin. The effect of human 7S γ-globulin on each of the mouse 7S γ-globulin components was investigated in the present work. Administration of human γ-globulin in large amounts caused a marked acceleration of the catabolism of mouse 7S γ_1-globulin, γ_{2a}-globulin, and γ_{2b}-globulin (Fig. 8). These findings in mice with low immunoglobulin levels were confirmed in normal mice (Table III). The effects of human γ-globulin on the catabolism of individual mouse myeloma proteins are similar to the effect on normal mouse 7S γ-globulin catabolism. Exogenous human 7S γ-globulin had no effect on mouse γ_1A-globulin metabolism (Table III), in accord with previous observations (5).

TABLE III

Effect of Exogenous (Human) γ-Globulin on the Catabolism of Mouse Immunoglobulins

	γ_{2a}		γ_{2b}			7S γ_1	γ_{1A}	
	Adj. PC-5	5563	MPC-31	MPC-37	MPC-11	MPC-25	SPC-1	MPC-40
NIH-WS (Control)	5.4*	4.8	3.0	2.0	2.6	4.0	1.0	1.0
Injected‡ NIH-WS	2.2	2.0	1.7	1.0	2.1	2.0	1.0	1.0
CVN or GF (Control)	10.2	11.8	1.8	2.0	3.3	14.0	1.1	1.1
Injected‡ CVN or GF	2.1	1.9	0.7	0.6	1.6	2.0	1.1	1.0

* Half-life in days.

‡ 10 mg human IgG (7S γ_2-globulin)/day/animal is maintenance dose.

DISCUSSION

Control of Catabolism.—Five immunoglobulin components of mice were found to have different catabolic properties.

The γ_{2a}-globulins had a fractional rate of catabolism of approximately 13 per cent/day in normal mice while the γ_{2b}-globulins were catabolized at almost twice this rate; *i.e.*, about 25 per cent per day. These observations were confirmed with several myeloma proteins, and the catabolic rates of the γ_{2a}- and γ_{2b}-protein groups did not overlap. The γ_{2a}- and γ_{2b}-myeloma proteins differ in other respects. The genetically determined Iga-1 isoantigens are present on γ_{2a}-globulins but not on γ_{2b}-globulins (8). The γ_{2a}-globulins sensitize guinea pig skin for reverse passive cutaneous anaphylaxis, which γ_{2b}-globulins will not do (18). The γ_{2a}- and γ_{2b}-globulins also differ in antigenic determinants which can be detected with rabbit antiserum (8). The catabolic differences observed in the present study are another parameter of difference between these molecules.

The 7S γ_1-myeloma protein was catabolized at a rate of about 17 per cent per day. The 7S γ_1-protein clearly differs in catabolic properties from the γ_{2b}-myeloma proteins. Before an exact comparison of 7S γ_1- and γ_{2a}-proteins can be made, however, additional myeloma proteins of each type would have to be studied. The 7S γ_1-globulins differ from both the γ_{2a}- and γ_{2b}-globulins in antigenic determinants (7) and in capacity to sensitize homologous (mouse) skin (18–20).

Prior studies of the turnover of I^{131}-labeled normal 7S immunoglobulins have shown a progressive change (flattening) of the decay curve; *i.e.*, a progressive reduction in the fractional rate of catabolism of the labeled protein (5, 16). This finding could be accounted for by the existence of molecules with different catabolic properties in the normal population. Those molecules with the more rapid rates of catabolism (shorter half-lives) would be removed most rapidly. On each succeeding day, there would be a progressively greater proportion of molecules with slower rates of catabolism among the remaining population of labeled 7S immunoglobulins. This would produce a flattening of whole body decay curves. The catabolic heterogeneity might have been artifactual due to molecular differences introduced during purification or radioactive labeling of the globulins. On the other hand, the changing curve of normal 7S immunoglobulin catabolism could have resulted because the normal population was heterogeneous in terms of catabolic rates. This last interpretation is supported by the present observations of catabolic heterogeneity of 7S immunoglobulins in the mouse.

The catabolic curves of normal IgG (7S γ-globulin) preparations in man also show a notable change (flattening) of the decay curve (1, 6) indicating that in man, as in the mouse, the normal IgG population is catabolically heterogeneous. Investigation of the four subclasses of human IgG (γ_{2a}, γ_{2b}, γ_{2c}, and γ_{2d}), which differ on the basis of specific heavy polypeptide chain features (21, 22) may be fruitful in this respect.

The evidence for metabolic heterogeneity in the normal 7S globulin population indicates that data obtained from studies of I^{131}-labeled normal 7S immunoglobulins represent mean values for mixtures of proteins. The finding of normal 7S immunoglobulin half-lives of about 4 days in normal mice and 10 days in low pathogen mice (5, 16) indicate that the short-lived 7S γ_{2b}-molecules may represent only a small part and γ_{2a}- and 7S γ_1-globulin molecules a larger part of the normal IgG (7S γ-globulin) population.

Differences in catabolism of several classes of 7S immunoglobulin have been emphasized in the preceding discussion. Similarities in control of catabolism also need to be emphasized.

The catabolic rates for γ_{2a}-, γ_{2b}-, and 7S γ_1-globulins showed a similar dependence on the serum level of 7S immunoglobulin. When the serum immunoglobulin levels were low, about 10 per cent of normal as in mice with little

exposure to pathogens, the half-time was prolonged and the fractional rate of catabolism was reduced to as low as 5 per cent per day for γ_{2a}-globulins and 7S γ_1-globulins (one-third of the normal rate). When all serum immunoglobulin levels were increased by hyperimmunization, the half-time for each 7S immunoglobulin was shortened. The fractional rate of catabolism was increased up to 30 per cent per day for γ_{2a}- and 7S γ_1-globulin (twice the normal rate). The γ_{2b}-globulin showed parallel changes at low and high immunoglobulin levels. Similar changes occurred when the serum 7S γ-globulin level was raised by the injection of human γ-globulin (IgG). These findings show that all three classes of 7S immunoglobulin had a similar catabolic relationship to the total serum level of 7S immunoglobulin.

The studies of catabolic control in low pathogen and hyperimmunized mice did not indicate whether each class of 7S immunoglobulin was responsive to the level of all 7S immunoglobulins or was sensitive only to the level of a single class of 7S immunoglobulin. This question was investigated by taking advantage of the selective immunoglobulin increases due to serum myeloma protein in mice with plasma cell tumors. Observations conducted in mice with high myeloma protein levels indicated that selective increase of one class of 7S immunoglobulin increased the catabolism of all three classes. This similarity indicated that catabolism was regulated by a property common to these classes of immunoglobulin. Other studies have shown that this is a property of the heavy polypeptide chain of the molecule (5).

The mechanism controlling the fractional rate of 7S γ-globulin catabolism remains to be identified. Brambell, Hemmings, and Morris (23) have proposed that some of the 7S γ-globulins become isolated from the general pool each day, and that at the time of this isolation some molecules become attached to specific receptors which protect these molecules (from catabolism) and return them undamaged to the general pool, whereas the unprotected molecules are degraded. At low serum γ-globulin levels, many protector sites would be available in relation to the total number of 7S immunoglobulin molecules and, thus, account for a low rate of catabolism (6, 16). At high serum γ-globulin levels, the protective sites would be relatively fewer and a greater fraction of 7S immunoglobulin would be catabolized (5, 6, 16). The present data are in accord with this hypothesis and indicate that the γ_{2a}-, γ_{2b}-, and 7S γ_1-globulin molecules may be protected from catabolism in a similar way.

The γ_1A (IgA, β_{2A})- and γ_1M (IgM, β_{2M})-globulins were notable for their rapid rates of catabolism and for their independence from the factors controlling 7S immunoglobulin catabolism. The fractional rate of catabolism was estimated at 55 per cent per day for IgA (γ_1A, β_{2A}) and 140 per cent per day for IgM (γ_1M). Catabolic measurements of these proteins presented major problems and the present data can only be regarded as approximations. The rapid rate of IgA catabolism (55 per cent per day) could have led to accumulation

of free I^{131} in the body and produced apparent half-times of survival that are greater than the true value (as well as falsely low estimates of the fractional rate of catabolism). There was, however, no evidence of an artifact from the shape of the whole body radioactivity decay curves. The basis for the two different macroglobulin antibody half-times observed (0.2 and 0.6 days) remains to be explained. Both half-times were found in at least two separate experiments. In any event, rapid rate of catabolism was characteristic of the IgM (γ_1M) molecules.

Rates of Synthesis.—The rates of synthesis for each of the immunoglobulin components were found to be of the same order of magnitude (Table IV). In

TABLE IV

Approximate Synthetic Rates for Immunoglobulins (mg/day/25 gm mouse)

	Normal mice	Hyperimmune mice	Low pathogen mice
7S γ_{2a}*: if 50 per cent	0.65	5.6	0.025
if 10 per cent	0.13	1.1	0.005
7S γ_{2b}*: if 50 per cent	1.15	9.2	0.078
if 10 per cent	0.23	1.9	0.015
7S γ_1	1.06	17.5	0.025
γ_{1A}(IgA, β_{2A})	0.64	4.5	‡
γ_{1M}(IgM, β_{2M})	1.4	7.7	‡

* Two calculations are given for 7Sγ_{2a}- and 7Sγ_{2b}-globulins based on the possibility that these represented 50 or 10 per cent of the total 7S γ_2-globulin population. The exact serum level of these two components was not determined.

‡ These components could not be detected in the serum of low pathogen mice. If the serum level was one-half of the minimal level that could be detected, the rates of synthesis would be about 0.005 mg/day for these proteins.

normal mice the IgM (γ_1M-globulin) is synthesized at about the same rate as the 7S γ_2- and 7S γ_1-globulins. The serum level of IgM (γ_1M), however, is normally only 10 or 20 per cent of the level of IgG (7S γ-globulins) because of the rapid rate of IgM catabolism.

The amount of IgA (γ_1A-, β_{2A}-globulins) synthesized daily is not fully reflected in the relative serum concentration (Table IV). The rapid rate of catabolism causes the serum level of IgA to be lower than the serum level of 7S γ_2- and 7S γ_1-globulins.

The rates of immunoglobulin synthesis in hyperimmunized mice are markedly increased, the data in Table IV showing 5- to 10-fold increases in rate of synthesis for all immunoglobulins. The data for IgA (γ_1A) synthesis in hyperimmunized mice (Table IV) is based on observations in C57BL and BALB/c mice which typically show higher IgA (γ_1A) levels (24) and probably have a correspondingly greater rate of IgA (γ_1A) synthesis than the white Swiss

(NIH-WS) mice used for the present studies. With this exception, the remaining values in Table IV are average values from observations in this study.

The observations in low pathogen (germfree or CVN) mice indicate the importance of antigen exposure in determining the production rate of immunoglobulins. Mice with a minimum of antigenic experience had low serum levels and very low rates of synthesis (Table IV). Many of the germfree mice available at the time of these studies evidently had been exposed to considerable amounts of antigen because they had relatively abundant serum 7S γ_2-globulin levels (Table I). The CVN-low pathogen mice, however, had low 7S γ_2-globulin levels, as did germfree mice investigated previously (16). The rates of γ_2- and

TABLE V

Estimates of Molecular Synthetic Rates (molecules of Immunoglobulin/day/25 gm mouse)

	Normal mice		Low pathogen mice (intact molecules)
	(Whole molecules*)	(6.6S units)	
7S γ_2(γ_{2a} + γ_{2b})	3.6×10^{15}	3.6×10^{15}	2×10^{14}
7S γ_1	4.2×10^{15}	4.2×10^{15}	1×10^{14}
γ_{1A}(IgA, β_{2A})	1.5×10^{15}	3.0×10^{15}	2×10^{13}‡
γ_{1M}(IgM, β_{2m})	8×10^{14}	4.0×10^{15}	5×10^{12}‡

* Based on amounts calculated in Table IV, and molecular weights of 150,000 for 7Sγ_2 and 7Sγ_1-globulins, 300,000 for γ_{1A}(IgA) and 900,000 for γ_{1M}(IgM), the latter two made up of 2 and 5 units respectively.

‡ Based on the assumption that serum concentration is ½ of the minimal amount of immunoglobulin detectable by the quantitative technics used. The actual rate of synthesis for these two proteins might be much less than these amounts but could not be more than twice the levels given above.

γ_1-globulin synthesis calculated in Table IV for low pathogen mice reflect the experience with this larger group of animals.

In the low pathogen mouse, the rates of immunoglobulin synthesis are much reduced (Table V). Even in the mouse with the lowest 7S γ_2-globulin levels, however, the calculated rate of 7S γ-globulin synthesis was 10^{13} molecules per day (16). This rate of synthesis in germfree mice is low in comparison to normal but is not low in absolute terms.

The rate of synthesis of IgA (γ_1A, β_2A) and IgM (γ_1M, β_2M) cannot be calculated in low pathogen mice because no serum level can be detected. This does not mean, however, that such proteins are not formed. The lower limit of detectibility of mouse IgA and IgM was estimated to be 0.01 mg/ml. If the serum level in low pathogen mice was one-half of this, *i.e.* 0.005 mg/ml, then it was calculated that the rate of synthesis of these proteins would be about 10^{13} molecules each day (Table V). These calculations are not meant to imply that these are the rates of IgA and IgM synthesis in low pathogen mice, but the calculation emphasizes that as many as 10^{13} molecules of these im-

munoglobulins can be synthesized per day without being detected in mouse serum.

SUMMARY

The metabolic properties of immunoglobulin were investigated by comparing five classes of mouse immunoglobulin. Three forms of 7S immunoglobulin had different rates of catabolism. The fractional rates of catabolism were found to be about 13 per cent per day for 7S γ_{2a}-globulin; 25 per cent for 7S γ_{2b}-globulin; and 17 per cent for 7S γ_1-globulin. Catabolism of the three classes of 7S γ-globulin (γ_{2a}, γ_{2b}, and γ_1) were prolonged at low serum 7S γ-globulin levels and accelerated at high serum 7S γ-globulin levels. Each of the 7S γ-globulin components was influenced by the serum level of the other mouse 7S γ-globulin components and by exogenously administered human 7S γ-globulin. They were not appreciably altered, however, by the serum level of IgA (γ_1A-, β_2A-globulin).

The progressively changing (longer) half-times observed in turnover studies of normal IgG (7S γ-globulin) may be caused by catabolic heterogeneity of normal 7S immunoglobulins which are immunochemically and catabolically related to γ_{2a}-, γ_{2b}-, and 7S γ_1-myeloma proteins.

These studies indicate that the 7S γ_{2a}-, 7S γ_{2b}-, and 7S γ_1-globulins share a common catabolic control mechanism. This mechanism is influenced by the serum level of each of these components, but is independent of the serum level of IgA (γ_1A-globulin) and probably is independent of IgM (γ_1M-globulin).

Catabolism of IgA (γ_1A-, β_2A-globulin) and IgM (γ_1M-globulin) was much more rapid than the catabolism of the 7S γ-globulins. The halftimes of the IgA and IgM were approximately 1.2 and 0.5 days respectively. The fractional rate of catabolism of IgA and IgM seemed to be independent of their serum concentration.

The rate of catabolism, as well as the rate of synthesis, was shown to play a major role in determining the serum level of each class of immunoglobulin.

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